

APPENDIX

IN THE SPECIFICATION:

Please amend the table on page 13 as follows:

Table 1 – Amino acid substitutions in the KNT mutant strains

SEQ ID NO:	Residue No.	2	17	25	57	61	62	66	75	91	94	102	112	116	117	159	188	190	196	197	198	199	203	206	207	211	220	234	238	246
1	WT*	N	H	D	M	E	A	H	V	Q	S	Q	S	L	E	T	S	S	V	K	Q	S	S	D	H	F	S	L	T	D
12	KT3-1		Y						A	R		R	P	F					L									V	A	
13	KT3-3			N	L	G	V		A		P	R			G			L								L				
14	KT3-5					G		Y	A	R	P	R										P						V		N
15	KT3-7	S				G		Y	A	R		R	P	F				T			L							V		
2	KT3-11	K				G		Y	A	R		R	P	F								P				L				
16	KT3-12						T	Y	A	R	P	K	T			L	G			R		P	P						A	
17	KT3-13					G		Y	A	R		R	P	F								P		V	Q					
18	KT3-15					G		Y	A	R		R	P	F								P		V	Q					
19	KT3-16					G		Y	A	R	P	K	P									P		V	Q		P			
20	KT3-19				L			Y	A	R	P	K	P									P		V	Q					
3	HTK	K			L	G	V	Y	A	R	P	R	P	F								P	P	V	Q	L	P	V	A	

IN THE CLAIMS:

1. A mutant kanamycin nucleotidyltransferase [having] comprising the sequence of SEQ ID NO:1 modified by at least one [or more] point mutation [mutations] selected from [a group consisting of] Met57Leu, [Ala62Val,] Ser94Pro, Ser203Pro, Asp206Val, His207Gln, Ser220Pro, Ile234Val and Thr238Ala [as against the protein comprising the amino acid sequence indicated by SEQ ID NO: 1], and having improved thermostability as compared to SEQ ID NO:1.

2. A mutant kanamycin nucleotidyltransferase with improved thermostability
[, wherein it comprises] as compared to SEQ ID NO:1, comprising the amino acid
sequence indicated by SEQ ID NO:2. [SEQ ID NO: 2.]

3. The mutant kanamycin nucleotidyltransferase according to claim 1,
comprising [wherein it comprises] the amino acid sequence indicated [in SEQ ID NO:
3.] by SEQ ID NO:3.

IN THE ABSTRACT:

It is desirable to have [To obtain a] selective markers [marker] suitable for
screening of thermophilic bacteria such as *Thermus thermophilus*. *T. thermophilus* are
good research materials for investigating the interrelation between enzyme structures
and functions since they are stable at extreme pH, crystallize easily and are easy-to-
handle.

[To provide a novel] Novel mutants of *Staphylococcus aureus* kanamycin
nucleotidyltransferase with markedly improved thermostability are disclosed, as well as
a selective marker using the same, and a screening method for thermophilic bacteria
such as [*Thermus*] *T. thermophilus* using said selective marker.

Table 1 – Amino acid substitutions in the KNT mutant strains

SEQ ID NO:	Residue No.	2	17	25	57	61	62	66	75	91	94	102	112	116	117	159	188	190	196	197	198	199	203	206	207	211	220	234	238	246	
1	WT*	N	H	D	M	E	A	H	V	Q	S	Q	S	L	E	T	S	S	V	K	Q	S	S	D	H	F	S	L	T	D	
12	KT3-1		Y						A	R		R	P	F				L										V	A		
13	KT3-3			N	L	G	V		A	P		R		G			L								L						
14	KT3-5					G		Y	A	R	P	R									P						V		N		
15	KT3-7	S				G		Y	A	R		R	P	F			T				L				L		V				
2	KT3-11	K				G		Y	A	R		R	P	F								P									
16	KT3-12						T	Y	A	R	P	K	T			L	G			R		P	P						A		
17	KT3-13					G		Y	A	R		R	P	F								P		V	Q						
18	KT-3-15					G		Y	A	R		R	P	F								P		V	Q						
19	KT3-16					G		Y	A	R	P	K	P									P		V	Q		P				
20	KT3-19				L			Y	A	R	P	K	P									P		V	Q						
3	HTK	K			L	G	V	Y	A	R	P	R	P	F								P	P	V	Q	L	P	V	A		

KT3-15 has the same mis-sense mutation as KT3-13. These two mutants, share three silent mutations, however KT3-13's two silent mutations and KT3-15's one mutation are mutually specific to each. Therefore, it is clear that these two mutants are distinct clones.